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A population-based case–control study of urinary bisphenol A concentrations and risk of endometriosis

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STUDY QUESTION: Is bisphenol A (BPA) exposure associated with the risk of endometriosis, an estrogen-driven disease of women of reproductive age?

SUMMARY ANSWER: Our study suggests that increased urinary BPA is associated with an increased risk of non-ovarian pelvic endometriosis, but not ovarian endometriosis.

WHAT IS KNOWN ALREADY: BPA, a high-volume chemical used in the polymer industry, has been the focus of public and scientific concern given its demonstrated estrogenic effects *in vivo* and *in vitro* and widespread human exposure. Prior studies of BPA and endometriosis have yielded inconsistent results and were limited by the participant sampling framework, small sample size or use of serum (which has very low/transient concentrations) instead of urine to measure BPA concentrations.

STUDY DESIGN, SIZE, DURATION: We used data from the Women's Risk of Endometriosis study, a population-based case–control study of endometriosis, conducted among female enrollees of a large healthcare system in the US Pacific Northwest. Cases were women with incident, surgically confirmed endometriosis diagnosed between 1996 and 2001 and controls were women randomly selected from the defined population that gave rise to the cases, without a current or prior diagnosis of endometriosis.

PARTICIPANTS/MATERIALS, SETTINGS, METHODS: Total urinary BPA concentrations were measured in 143 cases and 287 population-based controls using single, spot urine samples collected after disease diagnosis in cases. Total urinary BPA concentration (free and conjugated species) was quantified using a high-performance liquid chromatography-mass spectrometry method. We estimated odds ratios (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression, adjusting for urinary creatinine concentrations, age and reference year. We also evaluated the association by disease subtypes, ovarian and non-ovarian pelvic endometriosis, that may be etiologically distinct.

MAIN RESULTS AND THE ROLE OF CHANCE: We did not observe a statistically significant association between total urinary BPA concentrations and endometriosis overall. We did observe statistically significant positive associations when evaluating total urinary BPA concentrations in relation to non-ovarian pelvic endometriosis (second versus lowest quartile: OR 3.0; 95% CI: 1.2, 7.3; third versus lowest quartile: OR 3.0; 95% CI: 1.1, 7.6), but not in relation to ovarian endometriosis.

LIMITATIONS, REASONS FOR CAUTION: Given the short elimination half-life of BPA, our study was limited by the timing of collection of the single urine sample, that occurred after case diagnosis. Thus, our BPA measurements may not accurately represent the participants' levels during the etiologically relevant time period for endometriosis development. In addition, since it was not feasible in this population-based study to surgically confirm the absence of disease, it is possible that some controls may have had undiagnosed endometriosis.

WIDER IMPLICATIONS OF THE FINDINGS: By using population-based data, it is more likely that the controls represented the underlying frequency of BPA exposure in contrast to prior studies that used for comparison control women undergoing surgical evaluation, where the

indication for surgery may be associated with BPA exposure. The significant associations observed in this study suggest that BPA may affect the normal dynamic structural changes of hormonally responsive endometrial tissue during the menstrual cycle, promoting the establishment and persistence of refluxed endometrial tissue in cases with non-ovarian pelvic endometriosis. Further research is warranted to confirm our novel findings in endometriosis subtypes that may be etiologically distinct.

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Introduction

Bisphenol A (BPA) has been the focus of heightened media attention and legislative actions given its high production volume, estrogenic properties demonstrated *in vivo* and *in vitro* and widespread human exposure (Calafat *et al.*, 2008; Chapin *et al.*, 2008; NTP, 2008). BPA is used to manufacture polymers, such as polycarbonate plastic, a clear hard plastic found in re-usable food and beverage containers and other consumer products, and used to produce epoxy resin that lines the inside of metal food and beverage cans and metal lids of glass food jars (EFSA. European Food Safety Authority, 2006). Adults are believed to be exposed to BPA primarily from the ingestion of food and beverages that have become contaminated by direct contact with BPA-derived products. Consistent with its extensive use, BPA has been detected in the urine of 92% of the US population (Calafat *et al.*, 2008).

Epidemiologic data on the human health effects of BPA are limited, particularly with regard to endocrine-related diseases in women of reproductive age. One such disease that may be affected by BPA exposure is endometriosis, an estrogen-driven condition characterized by the presence of endometrial glands and stroma outside of the uterus, usually within the peritoneal cavity. This chronic, serious condition is associated with substantial morbidity including chronic, severe pelvic pain, dysmenorrhea, dyspareunia and infertility (Nisolle and Donnez, 1997). Although the true population prevalence of endometriosis is unknown given that surgery is required for definitive disease diagnosis, data from population-based studies suggest that endometriosis affects 6–11% of US women of reproductive age (Houston *et al.*, 1987; Missmer *et al.*, 2004; Weuve *et al.*, 2010; Buck Louis *et al.*, 2011). Prevalences as high as 43 and 48% have been reported for select groups of symptomatic women undergoing surgery for infertility and pelvic pain, respectively [as cited in Sangi-Haghpeykar and Poindexter (1995)].

Two prior epidemiologic studies examining the relationship between urinary BPA concentrations and endometriosis reported no statistically significant association (Itoh *et al.*, 2007; Buck Louis *et al.*, 2013). However, these studies were limited either by the restriction of the study populations to women undergoing surgical evaluation (Itoh *et al.*, 2007; Buck Louis *et al.*, 2013), the indication for which may be associated with BPA exposure, or by results based on just 14 women who were diagnosed with endometriosis within a screened population cohort of 127 women (Buck Louis *et al.*, 2013). A third study reported the suggestion of a positive association but was additionally limited by the use of serum instead of urine to measure concentrations of BPA, a non-persistent chemical that has very low or transient concentrations in blood (Cobellis *et al.*, 2009; Koch and Calafat, 2009; Calafat *et al.*, 2013). In the present analyses, we explored the association between urinary BPA concentrations and endometriosis, outside of the surgical setting, using data from a large, population-based case–control study. We also explored BPA concentrations in relation to subtypes of endometriosis, ovarian endometriosis and non-ovarian pelvic endometriosis, that may be etiologically distinct and may have a different relationship with BPA. It has been postulated that peritoneal endometriosis develops from endometrial tissue refluxed during menses that implants and persists in the peritoneal cavity whereas endometriomas may develop from another etiologic pathway, such as metaplasia of the invaginated celomic epithelium (Nisolle and Donnez, 1997).

Materials and Methods

Study design and population

We used data from the Women's Risk of Endometriosis (WREN) study and archived urine samples collected from a subset of WREN participants in an ancillary study, described elsewhere (Upson *et al.*, 2013a,b). Briefly, WREN, a population-based case–control study of endometriosis, was conducted among women aged 18–49 years who were enrollees of Group Health (GH), a large integrated healthcare system in the US Pacific Northwest. Cases ($n = 340$) were women first diagnosed with endometriosis (International Classification of Disease 9th Revision diagnostic codes 617.0–617.5, 617.8–617.9, excluding adenomyosis) between 1 April 1996 and 31 March 2001, with medical record confirmation of direct surgical visualization of endometriosis; 48% of cases had pathology-confirmed endometriosis (Marino *et al.*, 2008, 2009). Controls ($n = 741$) were women identified from computerized GH enrollment databases, without a current or prior diagnosis of endometriosis, frequency matched to cases in 5-year age groups. WREN study activities entailed participation in a structured, in-person interview that occurred after case diagnosis, eliciting detailed reproductive, contraceptive, medical and family history as well as lifestyle behavior information, prior to a reference date (Marino *et al.*, 2008, 2009). For cases, the reference date was the date of first GH visit for symptoms leading to endometriosis diagnosis; controls were assigned reference dates

corresponding to the distribution of reference dates among cases. Given the suggested role of endogenous hormones and endometrial tissue in the development of endometriosis, the eligibility criteria for cases and controls in the WREN study included premenopausal status, intact uterus and at least one ovary. In addition, cases and controls must have been enrolled in GH at least 6 months prior to the reference date. In the present analyses, we restricted cases to those with definite or possible endometriotic disease, defined as endometriosis with evidence of tissue invasiveness or interference with normal physiologic processes (Holt and Weiss, 2000).

As previously described, 169 cases and 343 controls were invited to provide a urine sample in the WREN study and 157 cases (93%) and 301 controls (88%) agreed (Upson *et al.*, 2013b). Depending on the volume of urine collected, each sample was aliquoted into one to four vials. For the present analyses, we selected the second vial that was available for 132 cases (84%) and 267 controls (89%) as the archived urine vial had not been previously used for laboratory analyses. To increase our sample size, we also included samples from 25 cases and 33 controls that had undergone at least 1 freeze/thaw cycle and laboratory analysis. We excluded six cases and six controls with a past history of surgically confirmed endometriosis discovered during the study interview, one case whose current endometriosis diagnosis was not confirmed surgically and five cases not meeting the definition of definite or possible endometriotic disease (Holt and Weiss, 2000). In addition, we were unable to use samples from one case and one control that had a low volume and samples from two controls that broke in transit to the laboratory. Thus, total urinary concentration of BPA was measured in samples from 144 cases and 291 controls in the present analyses (Supplementary data, Fig. S1).

Ethical approval

Institutional review board approval for the conduct of this study was received from the Fred Hutchinson Cancer Research Center. Each participant provided written informed consent before enrollment and participation.

Urine sample collection and total BPA measurements

A single, spot urine sample was collected from a subset of WREN participants in years 2001 and 2002 using a BPA-free polypropylene container. The samples were collected as part of the study activities at a location of the participant's choice; they were not collected in conjunction with medical care-related procedures. Among cases, sample collection occurred 6 months to 5.8 years after the date of endometriosis diagnosis (median, 3.4 years). The samples were refrigerated immediately after collection and aliquoted into BPA-free 30 ml flint glass vials with Teflon screw caps at the Fred Hutchinson Cancer Research Center Specimen Processing Laboratory. The urine specimens were frozen within 6 min to 8 h 10 min after collection (median 1 h, 24 min) and stored at -20°C until transport to the Institute for Preventive and Occupational Medicine in Bochum, Germany for laboratory analyses. Total urinary BPA concentration (free and conjugated species) was quantified using a high-performance liquid chromatography-mass spectrometry method (Koch *et al.*, 2012). The limit of quantification (LOQ) was 0.1 μg/l. To assess external contamination and degradation, samples ($n = 10$) with total BPA concentrations >10 μg/l ($>$97th percentile of concentrations) were additionally analyzed for free, or unconjugated, BPA. Free BPA was quantified using the same method as for total BPA, excluding the enzymatic hydrolysis step. The concentration of free BPA was $<$LOQ for seven samples and for the other three samples with free BPA $\geq$LOQ, the percentage of free BPA ranged from 0.56 to 2.16% of total BPA, indicating that sample contamination and/or degradation were not appreciable. Internal laboratory control procedures included analyzing quality control samples at two concentration levels, standards and reagent blanks in each batch of samples. The laboratory staff were blinded with regard to the case status of specimens and the inclusion of specimens for external quality assessment. We included a pooled sample and a duplicate sample in each batch to monitor the interbatch and intrabatch reliability. There was strong agreement for total BPA concentrations within batches, with an intraclass correlation coefficient (ICC) of 97%. The interbatch reliability among pooled samples was lower, as the coefficient of variation was 29%. Given the concern for external contamination and degradation among samples from 25 cases and 33 controls that had been previously used for laboratory analysis, we evaluated the within-person reliability between pairs of samples for 24 participants who had donated multiple vials of urine at the time of sample collection, including one vial that was previously analyzed and one that had never been analyzed. The ICC for the paired samples was 77%, indicating excellent reliability (Rosner, 2011), so total urinary BPA concentration data for these additional 58 WREN participants were included in our analyses. These analyses excluded one case and three control samples for which the laboratory was unable to obtain reliable peaks (matrix interferences) and that were not analyzable for total BPA. Urinary creatinine concentration was measured by means of the Jaffe reaction method (Taussky, 1954) with an analytic LOQ of 10 mg/l. One control sample with a creatinine concentration $>$300 mg/dl was excluded as high concentrations of creatinine may indicate dehydration in the participant, which may alter renal elimination of BPA (Barr *et al.*, 2005). Data on 143 cases and 287 controls were used in the present analyses.

Statistical analyses

We summarized the distribution of creatinine-uncorrected and creatinine-corrected total urinary BPA concentration by case status using the median and interquartile range (IQR). Creatinine-corrected total urinary BPA concentration was only used for descriptive purposes and was estimated by dividing the total urinary BPA concentration (μg/l) by the creatinine concentration (mg/dl) and multiplying by 100.

To investigate the relationship between total urinary BPA concentrations and endometriosis, we estimated the odds ratio (OR) and 95% confidence interval (CI) using unconditional logistic regression. Given that the exposure–disease relationship for an endocrine disrupting chemical may not be linear monotonic, we categorized total urinary BPA concentrations into quartiles to allow for a flexible exposure–disease model (Birnbaum and Jung, 2011). The quartiles were determined using the distribution among controls and were modeled as a set of indicator variables, with the lowest quartile serving as the referent. We adjusted for natural logarithm-transformed urinary creatinine (continuous), age ($\leq$19, 20–24, 25–29, 30–34, 35–39, 40–44, 45–49 years) and reference year (1995, 1996, 1997, 1998, 1999, 2000, 2001) by including these variables in the logistic regression model. These adjustment variables were selected *a priori* using a directed acyclic graph, informed by prior studies on sources of BPA exposure and risk factors for endometriosis (Supplementary data, Fig. S2) (Greenland *et al.*, 1999; Hernan *et al.*, 2002). We also decided *a priori* to repeat the analyses with additional adjustment for education ($<$high school, high school graduate, some college, college graduate, post graduate), alcohol consumption (never, former, current), smoking status (never, former, current) and race (non-Hispanic white, non-Hispanic black, non-Hispanic Asian/Pacific Islander, other/Hispanic) to evaluate if the ORs changed substantially with the inclusion of these variables, given the uncertainty of the relationship between these covariates and the exposure and outcome. To test the trend across quartiles of total concentration of BPA, we created a continuous variable by assigning values equal to the median quartile concentration among controls to participants in each exposure category. We included the variable in the adjusted logistic regression model and interpreted the accompanying *P*-value. We also evaluated the association between total urinary BPA concentrations and endometriosis by disease subtype, namely ovarian endometriosis ($n = 75$ cases) and non-ovarian pelvic endometriosis ($n = 68$ cases), as these disease subtypes may be etiologically distinct (Nisolle and Donnez, 1997). Women with surgical visualization of

endometriosis at both ovarian and non-ovarian sites were included among cases with ovarian endometriosis.

We conducted an exploratory analysis based on our hypothesis that controls restricted to women undergoing surgical evaluation may have altered BPA concentrations. Using the Wilcoxon rank sum test, we compared the distribution of BPA concentrations between controls with and without a history of laparoscopy or laparotomy and between controls with and without a history of tubal ligation, infertility testing, fibroids and ovarian cysts.

Statistical analyses were conducted using STATA 12.0 (StataCorp, College Station, TX, USA). The significance level of $\alpha = 0.05$ was used in all analyses. Since the exclusion of samples with non-detectable total BPA concentrations may result in biased estimates (Uh *et al.*, 2008), samples with missing values for total BPA (below the LOQ) were substituted using the value of the LOQ divided by the square root of two (LOQ/$\sqrt{2}$) (Lubin *et al.*, 2004). Under 8% of samples had values below the LOQ, thus the choice of value selected for substitution did not affect our determination of the median and IQR used to summarize the distribution of BPA, and samples with values <LOQ were included in the lowest quartile category.

Results

Among WREN participants with measured total urinary BPA concentrations, descriptive analysis of the group characteristics shows that a greater percentage of cases than controls were of Hispanic ethnicity, had completed college or post graduate education, were current consumers of alcohol, nulliparous and had greater concentrations of urinary creatinine concentrations (Table I). The distribution of characteristics among this subsample of WREN participants with laboratory measurements was generally similar to that found in the parent WREN study (data not shown) (Upson *et al.*, 2013b). In addition, the demographic characteristics of controls in this subsample by and large reflected those of the general population in the surrounding Puget Sound area (Saunders *et al.*, 2005).

Total BPA was detected in 92.1% of samples, and the distribution was right-skewed. For cases and controls, respectively, the median creatinine-uncorrected total BPA concentrations (μg/l) were 1.02 (IQR: 0.43–2.12) and 0.86 (IQR: 0.36–2.01), and the median creatinine-corrected total BPA concentrations (μg/g) were 1.32 (IQR: 0.79–2.21) and 1.24 (IQR: 0.65–2.54).

We did not observe a statistically significant association between total urinary BPA concentrations and endometriosis overall, adjusting for age, reference year and natural logarithm-transformed urinary creatinine, although the ORs across quartiles suggested a positive association (Table II). When evaluating total urinary BPA concentrations in relation to non-ovarian pelvic endometriosis, we observed statistically significant positive associations when comparing the second and first quartiles (OR 3.0; 95% CI: 1.2, 7.3) and third and first quartiles (OR 3.0; 95% CI: 1.1, 7.6) (Table III). The associations between total urinary BPA concentrations and ovarian endometriosis were not statistically significant and differed in direction by quartile. None of the tests of trend were statistically significant ($P > 0.05$). We observed generally similar results in analyses additionally adjusted for education, alcohol consumption, smoking status and race (Tables II and III).

We observed higher concentrations of BPA among controls with a history of laparoscopy or laparotomy ($n = 31$) compared with other controls, although the difference in distribution was not statistically significant (Supplementary data, Table SI). Our data also suggested that women who reported having had a tubal ligation ($n = 44$) had lower

Table I Characteristics of WREN participants with urinary total BPA measurements, GH, 1996–2001 [*n* (%)].

Characteristic	All types of endometriosis	
	Cases ($n = 143$)	Controls ($n = 287$)
Age (years)		
17–24	9 (6.3)	19 (6.6)
25–34	29 (20.3)	55 (19.2)
35–44	64 (44.8)	139 (48.4)
45–49	41 (28.7)	74 (25.8)
Race		
White	123 (86.0)	241 (84.0)
Black	6 (4.2)	16 (5.6)
Asian/Pacific Islander	11 (7.7)	21 (7.3)
American Indian/Aleut/Eskimo	2 (1.4)	2 (0.7)
More than one race	1 (0.7)	7 (2.4)
Ethnicity[a]		
Hispanic	10 (7.0)	7 (2.5)
Non-Hispanic	133 (93.0)	279 (97.6)
Income[a] (US $)		
<35 000	43 (30.7)	73 (26.1)
35 000–69 999	62 (44.3)	128 (45.7)
≥70 000	35 (25.0)	79 (28.2)
Education		
<HS	5 (3.5)	6 (2.1)
HS graduate	23 (16.1)	53 (18.5)
Some college	42 (29.4)	120 (41.8)
College graduate	44 (30.8)	66 (23.0)
Post graduate	29 (20.3)	42 (14.6)
Cigarette smoking		
Never	81 (56.6)	160 (55.8)
Former	31 (21.7)	74 (25.8)
Current	31 (21.7)	53 (18.5)
Alcohol use[a]		
Never	41 (28.9)	96 (33.5)
Former	20 (14.1)	60 (20.9)
Current	81 (57.0)	131 (45.6)
BMI (kg/m^2)[a]		
<18.5	2 (1.4)	7 (2.5)
18.5 to <25.0	76 (53.2)	144 (50.9)
25.0 to <30.0	30 (21.0)	71 (25.1)
≥30.0	35 (24.5)	61 (21.6)
Parity		
Nulliparous	64 (44.8)	87 (30.3)
Parous	79 (55.2)	200 (69.7)
Urinary creatinine (mg/dl) median (IQR)	85.5 (33.1, 131.1)	76.0 (34.1, 131.3)

HS, high school; IQR, interquartile range.
[a]Numbers may not add to the column total due to missing data.

Table II Results from unconditional logistic regression analyses for the relationship between urinary total BPA and endometriosis, GH, 1996–2001.

Total BPA concentrations (μg/l urine)	Cases (n = 143) n (%)	Controls (n = 287) n (%)	aOR[a] (95% CI)	aOR[b] (95% CI)
Quartiles				
≤0.364	31 (21.7)	72 (25.1)	1.0	1.0
>0.364–0.863	33 (23.1)	72 (25.1)	1.2 (0.6, 2.2)	1.3 (0.7, 2.4)
>0.863–2.01	40 (28.0)	72 (25.1)	1.5 (0.8, 3.0)	1.6 (0.8, 3.2)
>2.01	39 (27.3)	71 (24.7)	1.5 (0.7, 3.1)	1.5 (0.7, 3.1)
P_{trend}[c]			P = 0.342	P = 0.433

aOR, adjusted OR; CI, confidence interval.
[a]OR adjusted for age, reference year and natural logarithm-transformed urinary creatinine.
[b]OR adjusted for age, reference year, natural logarithm-transformed urinary creatinine, education, alcohol consumption, smoking status and race.
[c]P-value for test for trend across quartiles.

Table III Results from unconditional logistic regression analyses for the relationship between urinary total BPA and endometriosis, according to subtype of endometriosis, GH, 1996–2001.

Total BPA concentrations (μg/l urine)	Ovarian endometriosis				Non-ovarian pelvic endometriosis			
	Cases[a] (n = 75) n (%)	Controls (n = 287) n (%)	aOR[b] (95% CI)	aOR[c] (95% CI)	Cases (n = 68) n (%)	Controls (n = 287) n (%)	aOR[b] (95% CI)	aOR[c] (95% CI)
Quartiles								
≤0.364	22 (29.3)	72 (25.1)	1.0	1.0	9 (13.2)	72 (25.1)	1.0	1.0
>0.364–0.863	11 (14.7)	72 (25.1)	0.5 (0.2, 1.2)	0.5 (0.2, 1.3)	22 (32.4)	72 (25.1)	3.0 (1.2, 7.3)	3.3 (1.3, 8.3)
>0.863–2.01	18 (24.0)	72 (25.1)	1.0 (0.4, 2.2)	1.1 (0.5, 2.5)	22 (32.4)	72 (25.1)	3.0 (1.1, 7.6)	2.9 (1.1, 7.6)
>2.01	24 (32.0)	71 (24.7)	1.5 (0.6, 3.4)	1.3 (0.5, 3.3)	15 (22.1)	71 (24.7)	1.7 (0.6, 5.0)	1.7 (0.6, 5.1)
P_{trend}[d]			P = 0.091	P = 0.178			P = 0.761	P = 0.751

[a]Women with surgical visualization of endometriosis at both ovarian and non-ovarian sites were included among cases with ovarian endometriosis.
[b]OR adjusted for age, reference year and natural logarithm-transformed urinary creatinine.
[c]OR adjusted for age, reference year, natural logarithm-transformed urinary creatinine, education, alcohol consumption, smoking status and race.
[d]P-value for test for trend across quartiles.

concentrations of BPA, while women with a history of ovarian cysts (n = 42) had higher BPA concentrations compared with other controls.

Discussion

In the present analysis, our data suggested a positive association between total urinary concentrations of BPA and endometriosis that may be limited to non-ovarian pelvic endometriosis.

It is plausible that BPA may affect the risk of an estrogen-driven disease, such as endometriosis, given the estrogenic properties demonstrated by the chemical. Prior to its use in the plastics industry, the estrogenic properties of BPA were investigated in animal studies in the 1930s (Dodds and Lawson, 1936). Since this time, BPA has been classified as a weak estrogen based on nuclear estrogen receptor binding studies, although recent *in vitro* research indicates that BPA may exhibit greater estrogenic potency when mediated by estrogen receptors outside the nucleus (Wetherill *et al.*, 2007). Several *in vivo* studies have demonstrated the adverse endocrine disruptive effects of prenatal BPA exposure on the developing female reproductive tract, including advanced puberty, as indicated by early vaginal opening (Honma *et al.*, 2002; Markey *et al.*, 2003) and early estrus (Howdeshell *et al.*, 1999), persistent estrus (Markey *et al.*, 2003), altered endometrial histomorphology (Schonfelder *et al.*, 2004) and the presence of endometriosis-like lesions in the adipose tissue surrounding the reproductive tract (Signorile *et al.*, 2010). With regard to the effect of adult BPA exposure, one *in vivo* study found that BPA antagonized the effect of estradiol on progesterone receptors in endometrial stroma and glands, inhibiting rather than inducing progesterone receptor expression and progesterone activity (Aldad *et al.*, 2011). This is in line with investigations into endometriosis pathophysiology that have suggested the disease process involves progesterone resistance from reduced progesterone receptor expression in endometriotic tissue, resulting in a lack of response to progesterone to oppose the effects of estrogen (Bergqvist and Ferno, 1993; Attia *et al.*, 2000). Another *in vivo* study reported hormonal balance disruption after BPA treatment in adult female rats, with an increased serum estradiol-to-testosterone ratio and a decreased conjugated to free estrogen ratio, suggesting that BPA may alter estrogen metabolism to produce a hyperestrogenic environment (Quignot *et al.*, 2012). In the present

analyses, we hypothesized that BPA may affect the normal dynamic structural changes of hormonally responsive endometrial tissue during the menstrual cycle, promoting the establishment and persistence of refluxed endometrial tissue and interfering with endogenous hormonal activity to alter endometriosis disease risk. The statistically significant associations that we observed when restricting cases to those with non-ovarian pelvic endometriosis support this hypothesis, as this disease subtype is believed to occur from refluxed menstrual tissue (Nisolle and Donnez, 1997).

In contrast to our study, the two prior studies that compared urinary BPA concentrations between women with and without surgically visualized endometriosis reported no statistically significant associations (Itoh *et al.*, 2007; Buck Louis *et al.*, 2013). One study selected menstruating women scheduled for laparoscopy or laparotomy at one of 14 clinical centers, regardless of the indication for surgery and included women undergoing tubal ligation (Buck Louis *et al.*, 2013). The other study recruited nulliparous women undergoing laparoscopy for the treatment of infertility (Itoh *et al.*, 2007). Although studies of surgical populations are convenient and have the benefit of confirming the absence of disease in controls, the findings from these studies may be difficult to interpret. As suggested by our exploratory analyses, BPA exposure may be associated with the indication for surgical evaluation, resulting in overall altered concentrations among surgical controls.

In the study by Itoh *et al.* (2007), the comparison of endometriosis Stages II–IV with Stages 0–I as per the American Fertility Society classification scheme may have further limited the authors' ability to detect a statistically significant association; Stage I endometriosis may include the deep endometriotic implants (Koninckx *et al.*, 1994) that were positively associated with BPA in our study. In WREN, population-based controls were randomly sampled directly from the same defined source population that gave rise to the surgically visualized endometriosis cases, and therefore more likely represented the underlying frequency of BPA exposure. This hypothesis is supported by the results of the population cohort in the Buck Louis *et al.* (2013) study, a relatively small population cohort of 127 women recruited from population-based databases and screened for endometriosis by magnetic resonance imaging (MRI). In that cohort, first diagnosis of primarily ovarian endometriomas was detected in 14 women, with an adjusted OR of 1.68 (95% CI: 0.96, 2.92) associated with a one standard deviation increase in log-transformed urinary BPA concentrations (Buck Louis *et al.*, 2011, 2013). Although MRI had limited ability to detect the presence of all forms of endometriosis in the Buck Louis *et al.* (2013) study and resulted in a different distribution of disease detected by subtype/site than our study, in which the diagnosis was made by surgical visualization, the non-significant adjusted OR estimates observed in both of these population-based studies suggest that BPA is positively associated with endometriosis overall. To our knowledge, ours is the first study to investigate BPA in relation to endometriosis by disease subtypes, namely surgically visualized ovarian and non-ovarian pelvic endometriosis, providing important new information about a subset of endometriosis that may be more susceptible to BPA exposure.

Similar to prior studies (Itoh *et al.*, 2007; Buck Louis *et al.*, 2013), the major limitation of our study was the measurement of BPA using a single urine sample collected after the onset of disease among cases, a median of 3.4 years after the diagnosis date (range, 6 months to 5.8 years). BPA is a non-persistent chemical with a urinary elimination half-life <6 h (Volkel *et al.*, 2002) and studies of multiple urinary BPA concentration measurements among primarily non-pregnant women taken over varying intervals up to 3 years have reported poor to fair reproducibility, with ICCs ranging from 0.14 to 0.43 (Nepomnaschy *et al.*, 2009; Braun *et al.*, 2012; Townsend *et al.*, 2013). Hence, it is possible that our BPA concentration measurements did not accurately represent participants' levels during the etiologically relevant time period for the development of endometriosis. Given the similar sampling conditions between cases and controls and the calendar years of sample collection in our study, any error would most likely be non-differential, however, resulting in findings that are an attenuation of the true risk. Urine samples were collected from WREN participants at the time of the study interview, outside of the endometriosis-care-related procedures, preventing case exposure to medical equipment containing BPA. In addition, urine samples were collected in years 2001–2002, prior to the first published studies of BPA and endometriosis (Itoh *et al.*, 2007), thus it is unlikely that women diagnosed with endometriosis would have modified their behavior to reduce dietary exposure to BPA. It is also important to note that despite the estimated short half-life of BPA, this chemical was detected in 92.1% of samples in our study. This supports the conclusions of others that BPA exposure, which is primarily through daily diet, is ubiquitous and continuous (Calafat *et al.*, 2008). Hence, in the absence of behavioral changes, BPA quantification from urine collection after endometriosis diagnosis is likely in the aggregate to fairly represent pre-diagnosis levels.

Our study may have also been limited by the presence of undiagnosed endometriosis among the population-based controls since it was not feasible to surgically confirm the absence of disease in this group. However, given the disease definition employed in our study, definite or possible endometriotic disease, that focuses on progressive disease with interference with normal physiologic function, the frequency of undiagnosed endometriosis among controls is likely to be low. Using data from a population-based study on the prevalence of undiagnosed chronic pelvic pain (9%) (Mathias *et al.*, 1996) and the frequency of Stages III and IV endometriosis among women with chronic pelvic pain (18%) (Gruppo italiano per lo studio dell'endometriosi, 1994), Holt and Weiss (2000) estimated the prevalence of undiagnosed endometriotic disease to be small, at $<2\%$ (Holt and Weiss, 2000). Even if the frequency of any presence of endometriosis among women with chronic pelvic pain reported in the latter study was used (45%) (Gruppo italiano per lo studio dell'endometriosi, 1994), the estimated prevalence of undiagnosed endometriotic disease would still be just 4%. This suggests that the associations we observed were not substantially biased by the presence of undiagnosed disease.

Several aspects of our study allowed us to more accurately estimate the association between BPA and endometriosis compared with prior studies. First, we used data that employed a population-based sampling framework, precluding the possibility of atypical control selection that can occur among populations restricted to women undergoing surgical evaluation for existing medical indications, as suggested by our exploratory analyses. Secondly, our sample collection procedures resulted in minimal external contamination of samples and degradation of conjugated BPA during storage, as indicated by the low percentage of free BPA. These procedures included freezing samples within $\sim$8 h of collection, avoiding the use of preservatives in urine (Longnecker *et al.*, 2013) and using samples the majority of which had not undergone a prior freeze/thaw cycle or laboratory analysis. Thirdly, the WREN study size and the extensive information collected in the study allowed for the separate analyses of possibly etiologically heterogeneous disease

subtypes (Nisolle and Donnez, 1997), improving the sensitivity of our study to detect a statistically significant association between BPA exposure and non-ovarian pelvic endometriosis.

In conclusion, our study suggests that exposure to BPA may increase the risk of non-ovarian pelvic endometriosis, assuming a single BPA measurement is representative of exposure during the etiologically relevant time window. Further research is warranted to confirm the findings of our study, especially our novel findings by subtype of endometriosis that may be etiologically distinct.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

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Authors' roles

V.L.H. and D.S. conceived, designed and acquired data in the parent study and K.U., S.S., A.J.D., V.L.H. substantially contributed to the conception and design of the present study. H.M.K. provided laboratory analysis and acquisition of laboratory data and K.U. conducted the statistical analyses and drafted the manuscript. All authors provided input on the interpretation of data and manuscript revisions critical for important intellectual content. All authors approved the final manuscript version for publication.

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Conflict of interest

The authors declare they have no actual or potential competing financial interests.

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